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SPECIES-SPECIFIC PHYSIOLOGICAL RESPONSE OF DINOFLAGELLATES TO QUANTIFIED SMALL-SCALE TURBULENCE[1]

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Turbulence has been shown to alter different aspects of the physiology of some dinoflagellates. The response appears to be species-specific and dependent on the experimental design and setup used to generate small-scale turbulence. We examined the variability of the response of three dinoflagellate species to the turbulence, following the same experimental design used by Berdalet (1992) on *Akashiwo sanguinea* (Hirasaka) Ge. Hansen et Moestrup (=*Gymnodinium nelsonii* G. W. Martin). In all experiments, turbulence was generated by an orbital shaker at 100 rpm, which corresponded on bulk average, to dissipation rates (ε, quantified using an acoustic Doppler velocimeter) of ≈2 $cm^2 \cdot s^{-3}$. Turbulence did not appreciably affect *Gymnodinium* sp., a small dinoflagellate. However, *Alexandrium minutum* Halim and *Prorocentrum triestinum* J. Schiller exhibited a reduced net growth rate (33% and 28%, respectively) when shaken during the exponential growth phase. Compared to the still cultures, the shaken treatments of *A. minutum* and *P. triestinum* increased the mean cell volume (up to 1.4- and 2.5-fold, respectively) and the mean DNA content (up to 1.8- and 5.3-fold, respectively). Cultures affected by turbulence recovered their normal cell properties when returned to still conditions. The swimming speed of the cells exposed to agitation was half that of the unshaken ones. Overall, the response of *A. minutum* and *P. triestinum* was similar, but with lower intensity, to that observed previously on *A. sanguinea*. We found no clear trends related to taxonomy or morphology.

Key index words: **cellular DNA content; dinoflagellates; net growth rate; orbital shaking; small-scale turbulence; swimming speed**

Abbreviations: **ε, turbulent kinetic energy dissipation rate; μ_T, net growth rate during the turbulent period; μ_{TS}, net postshaking growth rate**

Field and laboratory studies on several dinoflagellate species have led to the hypothesis that this group of phytoplankton is especially sensitive to small-scale turbulence (Wyatt and Horwood 1973, Estrada et al. 1987, Peters and Marrasé 2000, last review by Berdalet and Estrada 2005). Dinoflagellates may proliferate in a diversity of habitats including frontal areas (Estrada and Blasco 1979, Smayda and Reynolds 2001), but the occurrence of dinoflagellate red tides appears to be favored by calm weather and water-column stability (Wyatt and Horwood 1973, Margalef et al. 1979, Pollingher and Zemel 1981, Berman and Shteiman 1998). In addition to the fact that water circulation patterns may favor the concentration, maintenance, and development of dinoflagellate populations, turbulence may have direct effects on cell biology. Laboratory data obtained using different species and a variety of experimental designs and setups show that dinoflagellate cells can be affected by small-scale turbulence to different degrees (Table 1). Reported alterations include changes in morphology (Zirbel et al. 2000) and swimming behavior (Estrada et al. 1987, Thomas and Gibson 1990, Chen et al. 1998, Karp-Boss et al. 2000), net growth rate decrease (Pollingher and Zemel 1981, Dempsey 1982, Thomas and Gibson 1990, 1992, Berdalet 1992, Berdalet and Estrada 1993, Juhl et al. 2000, Juhl and Latz 2002, Havskum 2003, Sullivan and Swift 2003, Havskum et al. 2005, Stoecker et al. 2006), cell death (Tuttle and Loeblich 1975, White 1976, Juhl and Latz 2002), cell-division inhibition (Pollingher and Zemel 1981, Berdalet 1992), increase of cell size (Berdalet 1992, Yeung and Wong 2003), changes in RNA and DNA cell content (Berdalet 1992) and cellular toxin content (Juhl et al. 2001), or bioluminescence stimulation (Anderson et al. 1988, Latz et al. 1994, Latz and Rohr 1999). On the whole, these studies suggest that small-scale turbulence interferes

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TABLE 1. Literature on effects of turbulence on different species of dinoflagellates.

Dinoflagellate species	Reference	Size (μm)	Setup	$\varepsilon(cm^2 \cdot s^{-3})$	Effect
Blastodiniales					
Pfiesteria piscicida	Stoecker et al. (2006)	12–20	Couette	0.09	⇓
Gonyaulacales					
Alexandrium catenella	Sullivan and Swift (2003)	30	Oscillating rod	10^{-4}, 1	=
Alexandrium fundyense	Juhl et al. (2001)		Couette cylinder	0.1	⇓
A. fundyense	Sullivan and Swift (2003)	30	Oscillating rod	1	⇑
Alexandrium minutum	Berdalet and Estrada (1993)	17	Oscillating grid	1	=
A. minutum	Chen et al. (1998)	[30]	Couette cylinder	164	⇓
Alexandrium tamarense	Sullivan and Swift (2003)	30	Oscillating rod	10^{-4}, 1	=
A. tamarense	White (1976)	[30–35]	Shaker		⇓⇓
Ceratium fusus	Sullivan and Swift (2003)	150–300	Oscillating rod	1	⇓
Ceratium tripos CL1	Sullivan and Swift (2003)	150–300	Oscillating rod	1	⇓⇓
C. tripos CL3	Sullivan and Swift (2003)	150–300	Oscillating rod	1	⇓⇓
C. tripos	Havskum et al. (2005)	200	Oscillating grid	0.05–1	=/⇓
Ceratocorys horrida	Zirbel et al. (2000)	[150–200]	Shaker	0.1–1	⇓
Crypthecodinium cohnii	Tuttle and Loeblich (1975)	[15–20]	Shaker		⊗
C. cohnii	Tuttle and Loeblich (1975)	[15–20]	Magnetic stirring or aeration		=
C. cohnii	Yeung and Wong (2003)	15–20	Shaker	0.1–≥1	⇓⇓
Fragilidium subglobosum	Havskum et al. (2005)	40–60	Oscillating grid	0.05–1	=/⇑
Lingulodinium polyedrum	Gibson and Thomas (1995)	[30–35]	Couette cylinder	0.73	⇓⇓
L. polyedrum	Juhl et al. (2000)	35	Couette cylinder	0.18	⇓
L. polyedrum	Juhl et al. (2000)	35	Shaker		⇓
L. polyedrum	Juhl and Latz (2002)	35	Couette	0.2, 1	⇓
				3.5	⊗
L. polyedrum	Juhl and Latz (2002)	35	Shaker		⇓
L. polyedrum	Sullivan and Swift (2003)	30	Oscillating rod	1	⇑
L. polyedrum	Sullivan et al. (2003)	30	Oscillating rod	10	⇓
L. polyedrum	Thomas and Gibson (1990)	[30–35]	Couette cylinder	0.2	⇓⇓
L. polyedrum	Thomas and Gibson (1990)	[30–35]	Shaker	200	⇓⇓
L. polyedrum	Thomas et al. (1995)	[30–35]	Couette cylinder	0.045	⇓⇓
Oxyrrhis marina	Havskum (2003)	16	Oscillating grid	1	⇓
Pyrocystis fusiformis	Sullivan and Swift (2003)	500	Oscillating rod	10^{-4}, 1	=
Pyrocystis noctiluca	Sullivan and Swift (2003)	[150–350]	Oscillating rod	1	⇓⇓
Scrippsiella lachrymosa	Smith and Persson (2005)	[10–17]	Magnetic stirring		⇓
S. trochoidea	Berdalet and Estrada (1993)	20	Shaker	2	⇓⇓, ⊗
Gymnodiniales					
Akashiwo sanguinea	Berdalet (1992)	36	Oscillating grid	1	⊗
A. sanguinea	Berdalet (1992)	36	Shaker	2	⇓⇓
A. sanguinea	Thomas and Gibson (1992)	[40–75]	Couette cylinder	0.011	⇓
A. sanguinea	Tynan (1993)	35	Couette cylinder	4.6	⇓⇓
Amphidinium carterae	Galleron (1976)	[10–15]	Shaker or bubbling		⊗
Gymnodinium catenatum	Sullivan and Swift (2003)	60	Oscillating rod	1	⇑
Gyrodinium sp.	Sullivan and Swift (2003)	60	Oscillating rod	10^{-4}, 1	=
Peridiniales					
Heterocapsa triquetra	Dempsey (1982)		Paddle	0.1	⇓
H. triquetra	Yeung and Wong (2003)	15–17	Shaker	0.1–≥1	⇓⇓
H. triquetra	Havskum and Hansen (2006)	18	Oscillating grid	0.05	⇑
Peridinium gatunense (as *P. cinctum* f. *westii*)	Pollingher and Zemel (1981)	50	Shaker		⇓
Scrippsiella trochoidea	Berdalet and Estrada (1993)	20	Shaker	>2	⇓⇓, ⊗
Prorocentrales					
Prorocentrum micans	Berdalet and Estrada (1993)	18	Shaker	2	⇓
P. micans	Tynan (1993)	[30–50]	Couette cylinder		⇓⇓
P. triestinum	Berdalet and Estrada (1993)	11	Shaker	>2	⇓

The organism size (approximate length in the longest axis) is the one indicated in each particular study. When the authors did not provide it, a cell-size range obtained from taxonomic guides (Tomas 1997) is indicated in brackets. The turbulence intensity values (ε) are the ones indicated by each study or those that can be calculated from the information provided; otherwise, the values are not noted here. Effects (sensu lato) are coded as no effect (=), increased (⇑), decreased (⇓), population growth inhibition (⇓⇓), and cell death (⊗). In addition to modifications in the population growth, each study focused on particular ecophysiological aspects that are not reflected in this table (modified from Berdalet and Estrada 2005).

with cell-division and life-cycle processes (including migration) of the studied species of dinoflagellates.

Apart from the negative effects listed above, increased or unmodified growth rates and cell sizes have been reported in other dinoflagellate species exposed to particular levels of small-scale turbulence (Table 1; Berdalet and Estrada 1993, Sullivan and Swift 2003, Sullivan et al. 2003, Havskum et al. 2005,

Havskum and Hansen 2006). Thus, the available studies suggest that the response (positive, negative, or indifferent) to small-scale turbulence should be species-specific and dependent on the turbulence intensity (Berdalet and Estrada 1993, Sullivan and Swift 2003). However, when trying to find the general trends, comparison among the available data has proved difficult. In part, this is due to the lack of quantification of the turbulence intensities applied (especially in the earlier experiments; Peters and Marrasé 2000). For example, agitation by an orbital shaker at 100 rpm caused immediate inhibition of the population development (cell abundance and net growth rate) of *Scrippsiella trochoidea* (F. Stein) Balech ex A. R. Loebl. grown in 10 mL tubes, but it had no significant effect in 4 L flasks containing 3 L of medium (Berdalet and Estrada 1993). Likely, the experimental turbulent kinetic energy dissipation rate (ε) and shear intensities were higher in the small tubes, but such estimations were not made. Differences among studies can also arise from the variety of species or clones studied and experimental approaches and setups (Berdalet and Estrada 1993, Sullivan and Swift 2003, Sullivan et al. 2003). For instance, in the study by Sullivan and Swift 2003, *Alexandrium tamarense* (M. Lebour) Balech was unaffected by the turbulence generated in a 20 L tank by vertically oscillating rods. In this study, the experimental ε intensities tested (estimated by acoustic Doppler velocimeter) were between 10^{-4} and 1 $cm^2 \cdot s^{-3}$. In contrast, complete growth inhibition was observed when *A. tamarense* was exposed to orbital shaking, probably at a much higher ε intensity (White 1976). Given that no turbulence estimations were made in the earliest study, and that the experimental setups were markedly different, inferring that growth inhibition of this species occurs above 1 $cm^2 \cdot s^{-3}$ should be exercised with caution. The experiments by Sullivan and Swift 2003 and Sullivan et al. 2003 also showed a sharp decrease (but no complete inhibition) in the division rate of *Lingulodinium polyedrum* (F. Stein) J. D. Dodge only at $\varepsilon \sim 10$ $cm^2 \cdot s^{-3}$, while at a lower range (10^{-4} to 1 $cm^2 \cdot s^{-3}$), division rate increased linearly with the logarithmic increase of ε intensity. In contrast, in this range of relatively low ε intensities, a marked reduction or even complete inhibition of *L. polyedrum* growth rate was observed in experiments using a Couette cylinder (Thomas and Gibson 1990, Juhl et al. 2000, Juhl and Latz 2002). Certainly, the volume and shape of experimental flasks establish constraints in the design of each experiment (duration, samplings, estimated parameters, etc.). Furthermore, they influence decisively the spectrum kind of water motion (shear) experienced by the cells, which could be more relevant than turbulence intensity (as measured by ε) in itself. Thus, the above mentioned examples point out the difficulties in comparing results from different experiments.

This study aimed to investigate whether the response to small-scale turbulence displayed by the red-tide-forming *A. sanguinea* was common to other dinoflagellate species when using the same experimental setup and design as Berdalet (1992). In that study, exposure of *A. sanguinea* (3 L cultures) to constant orbital shaking (100 rpm) resulted in the inhibition of the net population growth and an increase of the mean DNA content and average cell volume up to 10 and 1.5 times, respectively, compared to still controls. Additionally, a variety of cell forms (different in size, shape, and nuclear morphology) appeared in the shaken cultures. When turbulence stopped, population growth resumed and cell properties returned to normal values. In contrast, using the same design and experimental setup (although with a simpler sampling), cell abundance of *Prorocentrum micans* Ehrenb. decreased, while that of *S. trochoidea* was not affected by agitation (Berdalet and Estrada 1993). In the present study, three species of different taxonomic orders, *Gymnodinium* sp., *Alexandrium minutum*, and *Prorocentrum triestinum*, were chosen based on their size, shape, and presence/absence of cell-wall characteristics and were subjected to the same experimental design as *A. sanguinea* in Berdalet (1992). In the first experiment, a small *Gymnodinium* sp. was selected to test whether the strong response exhibited by *A. sanguinea* also occurred in smaller dinoflagellates. To investigate whether the response could be influenced by the presence of thecal plates or by the cell shape, we chose two different armored dinoflagellates for the second experiment: the ovoid *A. minutum*, with an intermediate size in between the two naked gymnodinioid species, and the teardrop-shaped *P. triestinum,* similar in size to the *Gymnodinium* sp. In addition to quantifying changes in cell abundance and net growth rate, morphology, cellular volume, and DNA content, we explored whether turbulence also affected swimming speed. Finally, in this study, we included the estimations of the turbulent kinetic energy dissipation rates (ε) in our experimental setup.

MATERIALS AND METHODS

Stock cultures. The characteristics of the different cultures used in these experiments are summarized in Table 2. Stock and experimental unialgal cultures were maintained in a temperature-controlled room under identical irradiance and culture media conditions. The cultures were kept at 20°C ± 1°C, on a 12:12 light:dark (L:D) cycle (light period starting at 8:00 a.m.). An irradiance of 150 μmol photon $\cdot m^{-2} \cdot s^{-1}$ was provided by the combination of Grolux (58 W; Sylvania, Erlangen, Germany) and cool-white (58 W; Philips, Eindhoven, the Netherlands) fluorescent lamps (in a 1:1 proportion). The cultures were grown in f/2-enriched seawater (salinity ~38 psu) without silicate addition (Guillard 1975). Oceanic seawater from the NW Mediterranean (85 km offshore of Barcelona) collected at a depth of 5 m was filtered through GF/F glass fiber filters (Whatman International Ltd., Middlesex, UK) and autoclaved at 121°C for 1 h in polycar-

TABLE 2. Characteristics of the cultures used in this study.

Organism	Order	Culture origin	Size (μm)	Volume (μm^3)	Shape	Thecal plates
Gymnodinium sp.	Gymnodiniales	M. Delgado	10.1 ± 0.1	546 ± 23	Ovoid	No
Prorocentrum triestinum	Prorocentrales	F. Gómez-Figueiras	11.7 ± 4.4	848 ± 45	Flattened drop	Yes
Alexandrium minutum	Gonyaulacales	F. Gómez-Figueiras	17.6 ± 5.3	2856 ± 77	Ovoid	Yes
Akashiwo sanguinea	Gymnodiniales	GSBL clone	37.0 ± 0.5	26330 ± 116	Ovoid-flattened	No

Data on *Akashiwo sanguinea* from Berdalet (1992). Size (μm) indicates the average (±standard error of the mean) value of the equivalent spherical diameter estimated by in vivo Coulter Counter measurements in still conditions (see Materials and Methods).

bonate bottles. Nutrient stocks were sterilized separately and added to the seawater under sterile conditions 24 h after autoclaving.

Experiments. To acclimate the cells, inoculations of the experimental vessels were performed after several transfers of exponentially growing stock cultures to new media. For all species, experiments were conducted in 4 L cotton-plugged, flat-bottom spherical flasks (Florence flasks; AFORA, Barcelona, Spain) with sampling tubes to allow sterile sampling. The flasks were filled with 3 L of culture medium. The number of flasks inoculated per species was limited by the fact that the orbital shaker (AOS from SBS, Rubí, Spain) could not hold more than four spherical flasks of such size and weight. For the first experiment with *Gymnodinium* sp., six experimental vessels were inoculated (two controls, four shaken). In the second experiment, which simultaneously tested *P. triestinum* and *A. minutum*, four flasks each were used (two controls and two shaken) for each species.

Samples for cell number, cellular volume, DNA content, and microscopic cell observations were taken at noon (12:00 p.m., i.e., 4 h after the onset of the light period) during the whole experiment, after carefully and gently swirling the flasks. The cultures were allowed to reach exponential phase prior to the start of the turbulent shaking, which covered different periods of the growth curve. In the first experiment, with *Gymnodinium* sp., two flasks were exposed to turbulence on day 7 after inoculation of the culture (T7, exponential phase), and two more flasks on day 10 (T10, late exponential phase), while two flasks (S) remained still throughout as controls. On day 14, all turbulence cultures returned to still conditions. In the second experiment, with *P. triestinum* and *A. minutum*, two flasks for each species were maintained under still conditions (S and ST), and two other flasks were exposed to turbulent shaking on day 4 (T and TS). One of the turbulent flasks (T) was continuously shaken until the end of the experiment, while the other was returned to still conditions on day 17 (TS). On that day, one of the two still flasks (ST) was exposed to turbulence for the remainder of the experiment. This second part of the experiment was thus conducted without replicates. In all cases, flasks were randomly chosen for the different treatments.

Turbulence intensity. To exactly match the experimental conditions described in Berdalet (1992), we used the same type of 4 L Florence flasks that were shaken at 100 rpm in the same orbital shaker employed in the previous study. The shaker had a displacement of 3.0 cm. This experimental setup provided the large volumes needed for multiple sampling of biological parameters (in particular, nucleic acid analysis), allowing a direct comparison between the two studies.

Turbulence was measured with a customized three-dimensional side-looking 10 MHz acoustic Doppler velocimeter probe (NDVlab from Nortek AS, Sandvika, Norway). The stem was detached from the signal conditioning module and held in place vertically with a hydraulic articulated arm (Hoffmann Group, Wetzlar, Germany; Fig. 1). A Florence flask was customized, widening its mouth gap to allow the insertion of the probe. The flask was firmly held in place on the shaker

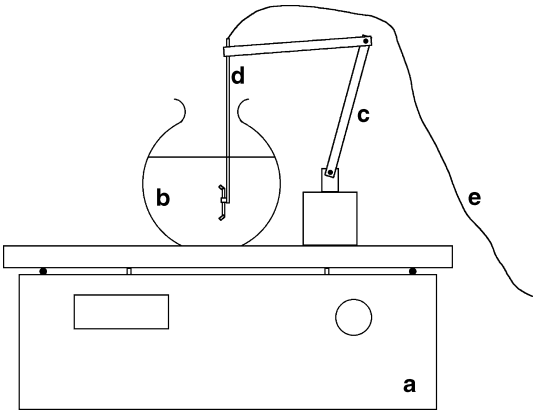

FIG. 1. Schematic representation of the setup used to measure turbulence within the Florence flasks on an orbital shaker table. Not drawn to scale. Shaker table (a), Florence flask modified at the mouth (b), articulated arm (c), velocimetry probe and stainless steel stem (d), and cabling to signal conditioning module (e).

table with bars and clamps. The articulated arm holding the probe was also firmly secured on the shaker table. Cabling reached loosely to the conditioning module ~0.5 m away from the shaker table. From there, a standard connection went to the data acquisition board in a personal computer. The turbulent kinetic energy dissipation rate (ε) was estimated from the –5/3 slope of the energy spectrum plot of each of the three-dimensional velocity time series. We used the linear regression method developed by Stiansen and Sundby (2001) that allows for filtering out the instrumental white noise. In all, 47 measurements were made at different points in the container and for rotation speeds from 60 to 125 rpm. Turbulence at a particular point in space was considered isotropic since dissipation rates calculated for each velocity component were similar, at least up to the minimum distance from the wall that was measured (1.5 cm). A multiple regression model with rotation frequency (ω, in Hz) and distance of the probe to both the wall (d, in cm) and the water surface (s, in cm) as explanatory variables was fitted to ε ($\log \varepsilon = -8.08 + 6.36\omega - 0.26d - 0.24s$; $n = 47$; adjusted multiple $r^2 = 0.89$). Rotation frequency was the most important explanatory variable (80% of the variance), while d and s accounted for the spatial variability within the flask. At 100 rpm, turbulence ranged from 0.27 $cm^2 \cdot s^{-3}$ in the middle of the container to 24 $cm^2 \cdot s^{-3}$ near the walls. At this rotation frequency, a bulk value of 2.01 $cm^2 \cdot s^{-3}$ was estimated by applying the above model to the equation of a truncated sphere, from the bottom of the flask to the height of the water level, at 1 mm spatial increments, and averaging the data.

Measurements. Cell number and cellular volume were estimated in vivo using a Multisizer Coulter Counter (Coulter Electronics Limited, Luton, England), immediately after

sampling. Two measurements were performed per sample. The precision (% coefficient of variation) of our cell abundance and cellular volume estimations were 1.5% and 4.7%, respectively. Samples (one per flask) for microscopic observations were fixed and stained with an acridine orange–formaldehyde mixture (Coats and Heinbokel 1982). A solution containing 200 μg · mL^{-1} acridine orange in concentrated 37% formaldehyde buffered with calcium carbonate was added in proportion 1:19 (v:v) to the sample. From the fixed samples, 1 mL was centrifuged (3500 rpm, 5 min), and most of the supernatant (~950 μL) was removed. From the concentrated pellet, a 10 μL aliquot was used to prepare slides for epifluorescence microscopy.

Net exponential growth rates, μ (t^{-1}), as defined by Guillard 1973, were calculated as the slope of the regression line of $\ln(N)$ versus time (t), where N was the estimated cell concentration. Only portions of the growth curve showing exponential increase were used for calculations. In the shaken cultures, we used the same approach to estimate what we refer to as the ''net growth rate during the turbulent period,'' μ_T, corresponding to the period of exposure to turbulence and the ''net post-shaking growth rate,'' μ_{TS}, for the period when the flasks were returned to still conditions. Such μ_T or μ_{TS} do not exactly match the time (or the physiological state) corresponding to the exponential growth rate phase of the controls, but they help compare the effects of turbulence on growth. For every treatment, a single regression line was calculated using data from the two replicates, after verifying the lack of significant differences between them. However, note that the estimation of μ_{TS} in the TS treatment (see the description of the experiments above) was performed on one unique flask.

On most sampling days, a sample was collected from every flask for the estimation of cell DNA content. DNA sampling was not performed on the first few days at the beginning of the experiment to avoid excessive water removal from the flasks in this period of low biomass. Samples for DNA determination were vacuum filtered (<100 mm Hg) through precombusted (450°C, 6 h) Whatman GF/F glass fiber filters and were subsequently stored in liquid nitrogen until analysis. DNA was measured following Berdalet (2002). Briefly, the filters were ground at 0°C in 100 mM Tris buffer (pH 8.0) containing NaCl (100 mM), $MgCl_2$ (0.9 mM), and $CaCl_2$ (0.9 mM). After incubation with RNase to digest RNA, DNA was quantified spectrofluorometrically using SYBR Green II (Molecular Probes Inc., Eugene, OR, USA). Calf thymus DNA (D-3664 from Sigma-Aldrich, St. Louis, MO, USA) was used as a standard. The precision of this method was 6.5% (coefficient of variation). The obtained DNA values were divided by the factor 4.18 to make them comparable (E. Berdalet, unpublished data) with a more recently developed method also based on SYBR Green II (Berdalet et al. 2005a,b).

The movement of *A. minutum* and *P. triestinum* from the TS and ST treatments was videotaped in a Palmer cell immediately after withdrawal of the samples from the incubation containers. After digitization, swimming velocities were calculated from the displacement of the cells and the time between frames (see Bartumeus et al. 2003 for more details). Twenty or more trajectories of each organism–time combination were analyzed. Randomly chosen cells were actively swimming 91% of the time on average, with values ranging from 76% to 97% of the time. No preferential swimming direction was observed, at least in the horizontal plane.

Statistical analyses. Comparison of treatments over time was performed using the paired sign test. This last test is analogous to a paired t-test but more generally used for small sets of data when normality cannot be assured (Motulsky 2003). Tests were conducted for the time courses of the three parameters analyzed in this study (cell number, mean cell volume, and mean DNA content). Growth rates were compared by testing for the heterogeneity of the slopes (analysis of covariance). Statistical analyses were conducted using Systat 5.1.2 for MacIntosh (SYSTAT Inc., Evanston, IL, USA).

RESULTS

First experiment—Gymnodinium *sp.* Until day 7 (when shaking started), the six flasks inoculated at the beginning of the experiment showed the same pattern over time in terms of cell abundance, cellular volume, and DNA content per cell (Fig. 2), without significant differences among replicates (nonparametric paired sign tests). Thereafter, from day 7 until the end of the shaking treatments, and even until the end of the experiments, lack of significant variability between the two replicates of each treatment over time (i.e., control, T7, and T10) was also observed (nonparametric paired sign tests).

Exponential growth occurred from days 4 to 14 in the controls. Turbulence did not appear to modify this pattern when applied either during the

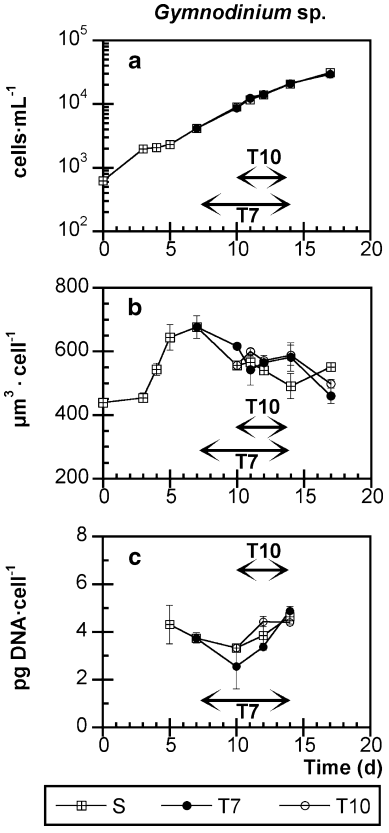

FIG. 2. Temporal changes in the biological parameters measured in *Gymnodinium* sp. cultures exposed to the different treatments. (a) Cell abundances in the cultures. (b) Cellular volume. (c) DNA content per cell. S: still (control); T7: turbulence applied between days 7 and 14; T10: turbulence applied between days 10 and 14. Arrows indicate the duration of the agitation period in every treatment. Data points are averages, and vertical bars are standard errors of the mean; n varied between six (until day 7, no turbulence applied) and two (two replicates per treatment once turbulence started).

7–14 d (T7) or the 10–14 d (T10) period (Fig. 2a). Table 3 shows the net growth rates estimated in all treatments during the 4–14 d period and also the net growth rate during the turbulent period, μ_T, corresponding to the shaking period only (i.e., days 7–14 for T7 and days 10–14 for T10). Although μ_T values were slightly lower than the rates estimated for the whole 4–14 d period, the differences with the controls were not significant in any case (Table 3). Turbulence did not significantly affect either cellular volume or DNA content (Fig. 2, b and c, respectively). Microscopic observation of the cells did not reveal any trend that could be related to a particular experimental condition. Returning the shaken flasks to still-water conditions on day 14 had no effect, and cells in all flasks had similar characteristics to the end of the experiment (Fig. 2).

Second experiment—A. minutum. The four inoculated flasks were highly similar in terms of cell number (1304.5 ± 52.9 cells $\cdot$ mL^{-1}), cellular volume (3106.0 ± 36.0 μm^3 $\cdot$ cell^{-1}), and DNA content (7.3 ± 0.4 pg $\cdot$ cell^{-1}; average ± standard error of the mean [SEM], $n = 4$, for the three parameters) on day 4, when two of them were exposed to shaking while the other two continued as controls (Fig. 3). Good replicability continued afterward, between days 4 and 17 of the experiment, within the two flasks of each treatment (i.e., S and ST [controls], or T and TS [shaken]; $P >> 0.1$ was obtained in all performed paired sign tests between the two replicates of each treatment and for the three parameters). The control populations of *A. minutum* grew exponentially from day 4 to day 11 at 0.33 ± 0.04 d^{-1} (Table 3; Fig. 3a). The typical cell observed in this period was almost spherical, measured 2762.0 ± 78.8 μm^3 ($n = 10$) in cell volume (Fig. 3b), had ~5.7 ± 0.7 pg ($n = 6$) DNA cell content (Fig. 3c), and had a characteristic U-shaped nucleus (Fig. 4, a and b). After day 17, the still culture (note that only one S flask remained as control) entered stationary phase, and large ovoid cells were observed (Fig. 4d). Unfortunately, it was not possible to identify whether this cellular phase corresponded to a nonmotile resting cyst resulting from the fusion of conjugating gametes (diploid, sexual) or to a temporary resting cyst (haploid, asexually formed by ecdysis). The proportion of these different cellular forms increased toward the end of the experiment, although the distribution of the cell-size classes computed by the Coulter Counter continued to be unimodal (not shown). The mean cell volume and the mean DNA content of the

TABLE 3. Net growth rates (μ, d^{-1}) estimated for each experimental condition of the three species studied.

Treatment	Days	μ	Error	r^2	P	T/S%
Gymnodinium sp.						
S (μ)	4–14	0.250	0.013	0.967		
T7 (μ_T)	4–14	0.248	0.013	0.964	0.515	99
T7 (μ_T)	7–14	0.232	0.010	0.983	0.409	93
T10 (μ_T)	4–14	0.245	0.016	0.946	0.294	98
T10 (μ_T)	10–14	0.197	0.016	0.955	0.320	79
Alexandrium minutum						
S (μ)	4–11	0.328	0.037	0.894		
T (μ_T)	5–17	0.108	0.005	0.976	0.009	33
TS (μ_{TS})	18–21	0.204	0.033	0.948	0.000	62
Prorocentrum triestinum						
S (μ)	4–10	0.485	0.020	0.988		
T (μ_T)	5–14	0.136	0.008	0.972	0.003	28
TS (μ_{TS})	18–25	0.223	0.007	0.997	0.000	46

In the case of the shaken treatments, μ refers to μ_T, the net growth rate during the turbulent period, or to μ_{TS}, the net postshaking growth rate (when the flask was returned to still conditions, see Materials and Methods). For all estimations of μ we indicate the period of the experiment (days) considered and the associated standard error and adjusted squared multiple r^2. P: degree of significance of the heterogeneity of the slope tests (analysis of covariance) run to compare μ_T or μ_{TS} with the corresponding μ of the control. A comparison between growth rates related to turbulent conditions (either shaking or postshaking) and still conditions is indicated as the T/S%.

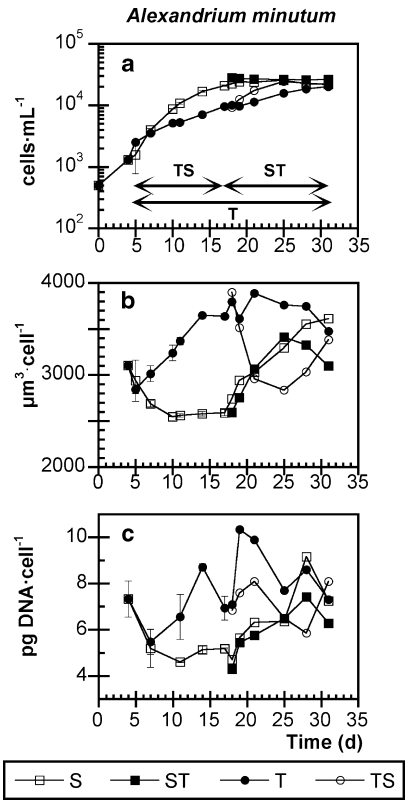

FIG. 3. Temporal changes in the biological parameters measured in *Alexandrium minutum* cultures exposed to the different treatments. (a) Cell abundances in the cultures. (b) Cellular volume. (c) DNA content per cell. S: still (control) during the whole experiment; ST: still conditions until day 17, and turbulence applied thereafter; T: turbulence started on day 17; TS: turbulence applied during days 4–17. Arrows indicate the duration of the agitation period in every treatment. Data points are averages, and vertical bars are standard errors of the mean; n was 4 (until day 4, no turbulence applied), 2 (between days 5 and 17, two control and two shaken flasks), and 1 (no replication after day 17 until the end of the experiment).

population increased during the stationary phase in the control (Fig. 3, b and c).

When flasks T and TS were shaken, their population development (in terms of cell numbers) was significantly lower than that of the controls (paired sign test, $P = 0.039$; Fig. 3a). Their μ_T (days 5–17) was significantly ($P = 0.009$) lower (~33%) than that of the still flasks (Table 3; Fig. 3a). Average cell volume and DNA content per cell increased significantly ($P = 0.039$ and $P = 0.021$, respectively) in the shaken cultures (Fig. 3, b and c), with maximum values (computed in flask T during days 18–28) ~140% (3760 ± 45 μm^3, $n = 5$) and 180% (10.1 ± 0.2 pg, $n = 2$), respectively, of the values measured during the exponential phase of the still controls. Microscopic observations revealed that the nuclei of the shaken cells lost their typical U-shape and appeared as a uniform globose mass mostly located in the epitheca (Fig. 4c). In addition, a subpopulation of large ovoid cellular forms similar to those observed during the stationary phase of the control cultures was observed (Fig. 4d). The presence of this subpopulation (identified also by the bimodal distribution of the cell-size classes computed by Coulter Counter) resulted in an increase in the mean cell volume. When shaking was stopped on day 17 in treatment TS, an immediate increase in cell number occurred (Fig. 3a), although the net postshaking growth rate, μ_{TS}, in this period (days 18–21) was still significantly ($P = 0.000$) lower (~62%) than that of the exponentially growing still cultures on days 4–11 (Table 3). Moreover, average DNA content per cell and cell volume decreased, and the size-class distribution became unimodal (not shown). Large numbers of small spherical cells with a bright U-shaped nucleus were observed (Fig. 4, a and b). However, the TS culture soon entered the stationary phase with a concomitant second increase in DNA content, cell volume (with the bimodal distribution of cell-size classes), and a second appearance of large ovoid cells (Fig. 4d). Shaking did not modify the growth or cellular characteristics of the ST culture when this still flask was placed on the shaker on day 17, after the culture had already entered the stationary phase (Fig. 3). No significant differences in the cellular volume or DNA content with flask S in stationary phase were detected. Indeed, at the end of the experiment, the four treatments were in stationary phase, with no significant differences among them regarding the three measured parameters.

The swimming velocity of the cells appeared to be affected by the previous turbulence treatment (Fig. 5a). Before day 17, cells removed from the ST flask (maintained under still conditions since the beginning of the experiment) moved approximately twice as fast as those obtained from the TS vessel (exposed to turbulence from days 4 to 17). Thereafter, when shaking stopped on day 17, the organisms from this TS treatment appeared to have recovered the speed range of the still conditions. Conversely, when the ST culture switched from stillness to turbulence on day 17 until the end of the experiment, the cells taken from it slowed down (Fig. 5a).

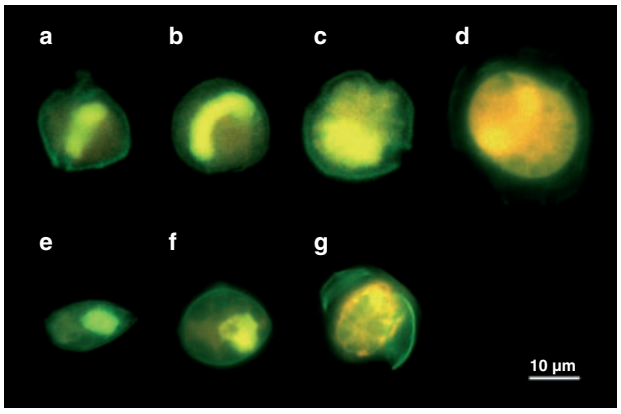

FIG. 4. Epifluorescence images of *Alexandrium minutum* (a, b, c, d) and *Prorocentrum triestinum* (e, f, g) cellular forms observed under the different treatments. *A. minutum*: cells under still conditions, with the characteristic U-shaped nucleus (a, b); cells observed in the turbulent flasks (c); and cells observed under the stationary phase in the still treatments and during the whole experiment under shaken conditions (d). *P. triestinum:* cells observed under still conditions (e), and cellular forms observed under turbulent conditions (f, g). Scale bar, 10 μm.

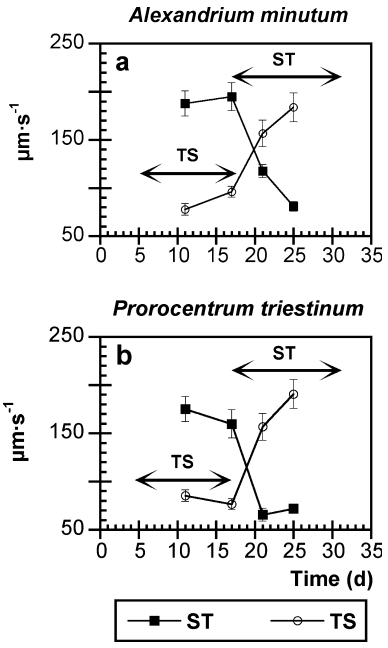

FIG. 5. Swimming velocities estimated in the TS and the ST flask of *A. minutum* (a) and *P. triestinum* (b). Symbols are the mean and standard errors of the velocity of at least 15 randomly chosen trajectories from the digitized clips. TS: turbulence applied during days 4–17; ST: still conditions until day 17, and turbulence applied thereafter. Arrows indicate the duration of the agitation period in each treatment.

Second experiment—P. triestinum. The four inoculated flasks were highly similar in terms of cell number (1505.3 ± 60.9 cells · mL^{-1}), cellular volume (1121.6 ± 13.5 $\mu m^3 \cdot cell^{-1}$), and DNA content (1.3 ± 0.2 pg · $cell^{-1}$; average ± SEM, $n = 4$, for the three parameters) on day 4, when two of them were exposed to shaking while the other two continued as controls (Fig. 6). As in the case of *A. minutum*, good replicability continued afterward, between days 4 and 17 of the experiment, within the two flasks of each treatment (i.e., S and ST [controls], or T and TS [shaken]; $P >> 0.1$ was obtained in all performed paired sign tests between the two replicates of each treatment and for the three parameters).

Exponential growth of the control populations occurred between days 4 and 10 at 0.49 ± 0.02 d^{-1} (Table 3; Fig. 6a). During this period, the average cell volume decreased, and the DNA cell content tended to increase. The values reached on day 10 (815.5 ± 36.5 $\mu m^3 \cdot cell^{-1}$, 2.4 ± 0.3 pg DNA · $cell^{-1}$, $n = 2$) remained fairly stable during the subsequent stationary phase until the end of the experiment (712.4 ± 20.7 $\mu m^3 \cdot cell^{-1}$, $n = 12$; 2.5 ± 0.1 pg DNA · $cell^{-1}$, $n = 12$).

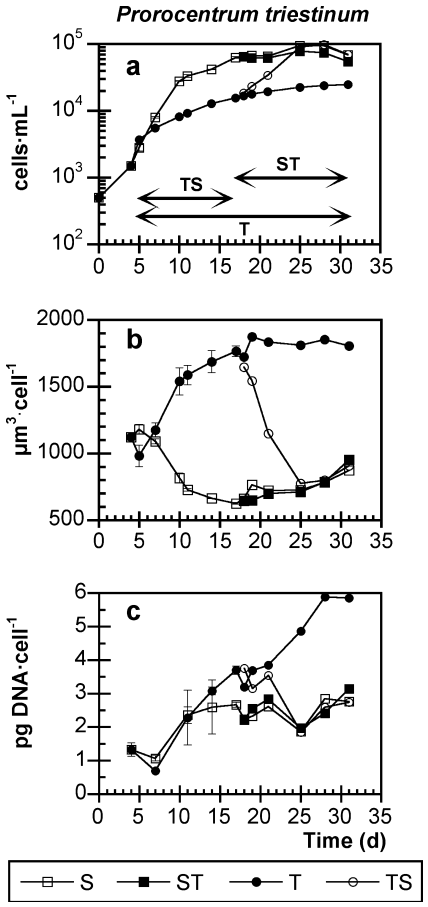

FIG. 6. Temporal changes in the biological parameters measured in *Prorocentrum triestinum* cultures exposed to the different treatments. (a) Cell abundances in the cultures. (b) Cellular volume. (c) DNA content per cell. S: still (control) during the whole experiment; ST: still conditions until day 17, and turbulence applied thereafter; T: turbulence started on day 17; TS: turbulence applied during days 4–17. Arrows indicate the duration of the agitation period in every treatment. Data points are averages and vertical bars are standard errors of the mean; n was 4 (until day 4, no turbulence applied), 2 (between days 5 and 17, two control and two shaken flasks), and 1 (no replication after day 17 until the end of the experiment).

In this species, changes induced by shaking were similar to those observed for *A. minutum*. Turbulence resulted in a rapid decrease in population development when applied early in the exponential phase. Significantly lower (paired sign test, $P = 0.039$) cell numbers were recorded in flasks T and TS during the shaking period (Fig. 6a), and the estimated μ_T values were also significantly ($P = 0.003$) lower (~28%) than those of the still flasks (Table 3). Cellular volume in the shaken cultures markedly increased until day 17 and stabilized thereafter until the end of the experiment. The values estimated in treatment T from days 17 to 31 (with a unimodal size-class distribution, not shown), 1816.2 ± 21.3 $\mu m^3 \cdot cell^{-1}$ ($n = 6$), were ~2.5 times that of the still cultures during the stationary phase. The cellular DNA content increased since the beginning of the shaking period in both T and TS treatments. At the end of the experiment, DNA values were ~5.3 times (5.9 ± 0.01 pg · $cell^{-1}$, $n = 2$) those estimated at the beginning (or 2.2 times those measured during the stationary phase) of the control flasks. Statistical analyses (nonparametric paired sign tests) showed that the differences in mean cell volume and mean DNA content between still and turbulent conditions were significant ($P = 0.006$ and $P = 0.039$, respectively). Rapid resumption of population development and recovery of ''normal'' cell properties occurred after shaking stopped in flask TS on day 17. However, in this period, the μ_{TS} was still significantly ($P = 0.000$) lower (~46%) than that estimated in the still treatments at the beginning of the experiment (Fig. 6a; Table 3). Finally, shaking caused a minor decrease in cell concentration when applied during the stationary phase of the culture (flask ST, day 17, Fig. 6a). Changes in cell volume or DNA content were also minor in the ST culture, which was shaken during the stationary phase (Fig. 6, b and c). No significant differences between S and ST treatments were detected for any of the three parameters.

Microscopic observations showed morphological changes likely associated with changes in cell volume for each treatment. Elongated, teardrop-shaped cells with a bright, round nucleus were observed in the still cultures throughout the whole experiment, independently of the culture growth phase (Fig. 4e). In contrast, swollen cells dominated in shaken cultures (Fig. 4, f and g). The change from shaken to still conditions in the TS flask was followed by a progressive decrease of swollen cells and the dominance of thinner cells. Swollen cells with a weakly stained nucleus appeared only on the

last day of the experiment in the ST treatment. Finally, turbulence affected the swimming speed of *P. triestinum* (Fig. 5b), with the same pattern observed for *A. minutum* (Fig. 5a).

DISCUSSION

The aim of this study was to test the effects of quantified small-scale turbulence on the population development (cell abundance and net growth rate) and cellular and nuclear characteristics of three different dinoflagellates, and then to compare those results to the published data for *A. sanguinea* (Berdalet 1992), obtained using the same experimental setup and design. While the agitation of the orbital shaker at 100 rpm (bulk average of ε, $\sim$2.01 $cm^2 \cdot s^{-3}$; range, 0.27–24 $cm^2 \cdot s^{-3}$) resulted in complete arrest of population development in the case of *A. sanguinea*, growth of *Gymnodinium* sp. was not affected. Further, *A. minutum* and *P. triestinum* showed intermediate responses, but there were also differences between these two species. In previous experiments (Berdalet and Estrada 1993), these two species had shown a certain degree of sensitivity (decreased population development) to small-scale turbulence generated by the same orbital shaker at 118–124 rpm on 10 mL tubes (*P. triestinum*) or by vertically oscillating grids (*A. minutum*). Our results corroborate that sensitivity of dinoflagellates to turbulence can vary with the species studied, the experimental setup, and the turbulence intensity, as also indicated by previous studies (Berdalet and Estrada 1993, Juhl et al. 2001, Sullivan and Swift 2003).

When exposed to turbulence, *A. minutum* and *P. triestinum* had lower net growth rates and cell numbers than control cultures under still conditions. This finding could be the result of a certain degree of cell mortality as observed in other studies (Berdalet 1992, Juhl et al. 2000, 2001, Juhl and Latz 2002), although we did not observe dead cells. Appropriate methods (Darzynkiewicz et al. 1994, Yenstch and Pomponi 1994) should be used in future studies to explore whether the turbulence, as generated in our experimental setup, causes cell mortality. Here, the decreased population development occurred simultaneously to the increase in mean cell size and mean DNA content, similar to the changes observed in *A. sanguinea* (Berdalet 1992), but with lower magnitude. In the case of *A. sanguinea* grown under turbulence, the marked DNA increase (up to 10 times with respect to the exponentially growing still culture) had been related to the existence of an important polylploid fraction of the population and/or to a transient arrest or an extension of the G2 + M phase of the cell cycle. This hypothesis could also apply to *P. triestinum*, where cellular DNA content of the turbulent treatments reached values up to 2.2–5.3 times that of the control ones. In the case of *A. minutum*, for which the highest increase was only $\sim$1.8 times, this change would indicate a high proportion of diploid organisms. Transient cell-cycle arrest at G1 has been described for *L. polyedrum* (Juhl and Latz 2002), *Crypthecodinium cohnii* Seligo, and *Heterocapsa* sp. (Yeung and Wong 2003). Unfortunately, the design and techniques used in our studies could not attribute the increase in DNA content per cell to a particular cellular phase or life-form (such as the big ovoid cells; Figs. 4, d and g). Again suitable microscopic or flow-cytometric techniques should help shed light on the interference of small-scale turbulence with the cell-cycle stages in dinoflagellates.

The increase in cell size and DNA content and the appearance of swollen cells, which can be interpreted as temporary (haploid) or resting (diploid) cysts (Wyatt and Jenkinson 1997), were observed in *A. minutum* and *P. triestinum* when the cells entered stationary phase in still conditions and also when cells were shaken. The entrance into stationary phase is often related to different nutrient limitations, and it can be accompanied in certain species by an increase in cyst production (Anderson 1998 and references therein). However, there is a wide diversity of exogenous and endogenous factors (studied also under a variety of natural and experimental conditions) that may trigger (sexual or asexual) cyst induction and germination (Anderson 1998, Garcés et al. 2002, Figueroa and Bravo 2005, Figueroa et al. 2005 and references therein) during the exponential or stationary growth phase. Turbulence could accelerate changes linked to other unknown limiting or stimulating factors that normally occur when cells enter the stationary phase. For instance, an increase in nutrient incorporation enhanced by turbulence (Karp-Boss et al. 1996, Peters et al. 2006) could subsequently accelerate the metabolic activity, which in turn could result in either the advancement of the limitation by some essential nutrient, or the production of a stimulating factor involved in the entrance into stationary phase or into cyst induction. Interestingly, Smith and Persson 2005 reported that turbulence prevented temporary cyst induction in *Scrippsiella lachrymosa* X. Gao et J. D. Dodge, which is in apparent contradiction to our data. It is too early to draw a clear conclusion about the interference of turbulence with cyst induction in *A. minutum* and *P. triestinum* from our present work. This aspect should be carefully considered in future studies.

Restoration of normal cell characteristics occurred when shaking stopped, indicating that calm conditions are preferable for cell division of certain species, such as *A. minutum*, *P. triestinum*, and *A. sanguinea*. However, this finding should be treated with caution given the lack of replication imposed by the shaker capacity constraints (see Materials and Methods). We based our confidence

on the consistency of the data obtained and on the lack of significant differences (nonparametric paired sign tests) between the two replicates during the first part of the experiment (see Results; Figs. 3 and 6). Net postshaking growth rates were lower than those observed in the exponential phase of still cultures. This could indicate a toll on physiological organization. Alternatively, lower growth rates could be expected at the cessation of shaking, because after the elapsed time, the cells were also closer to the stationary phase than at the beginning of the experiment. Note that turbulence did not completely inhibit population development or cause massive cell death. During the shaking period, cells managed to survive and even increase in cell numbers, thus consuming the existing nutrients and/or stimulating the production of inhibitory compounds (as suggested above) and progressing from a exponential to a stationary physiological phase. Overall, the speed with which normal growth resumed reflects how fast physiological changes occur when cells are returned to nonturbulent conditions.

The swimming speed of the cells taken from turbulent conditions was half that of the control ones for *A. minutum* and *P. triestinum*. Although our estimations were made on organisms withdrawn from the incubation containers, the trends were similar to the ones obtained from direct measurements on *A. minutum* swimming in Couette cylinders made by Chen et al. (1998). In their experiment, the mean value for the swimming velocity in unrotated cultures was $\sim$350 $\mu m \cdot s^{-1}$, compared to 170 $\mu m \cdot s^{-1}$ for cells in a culture whose growth was highly inhibited by exposure to 60 rpm (corresponding ε of $\sim$164 $cm^2 \cdot s^{-3}$) during 5 d. Lower swimming speeds could be explained as an adaptation of the cells when the water motion is strong enough to alter their regular motility behavior or to overcome any advantages due to active swimming. At least under certain conditions of water motion, it would be advantageous for the cells to reduce their own swimming speed and perhaps save a surplus of energy potentially useful for other metabolic investments. Contrasting observations were reported by Sullivan et al. 2003 for *Alexandrium catenella* (Whedon et Kof.) Balech. This species increased both the frequency of chain formation and chain length when grown under high-turbulence conditions ($\varepsilon \sim 3$ and 10 $cm^2 \cdot s^{-3}$). These two phenomena resulted in increased swimming speeds over that of single cells (and shorter chains) in *Gymnodinium catenatum* L. W. Graham and *Alexandrium affine* (H. Inoue and Y. Fukuyo) Balech (Fraga et al. 1989). The two studies speculated that higher swimming speeds of long chains may allow the cells to find a preferable area of the water column while swimming away from turbulence and shear. Preferential swimming orientation of both single cells and chains in response to turbulence was observed by Karp-Boss et al. (2000). Overall, apart from differences that may originate from different approaches and species, the alteration of swimming behavior suggests that exposure to certain levels of turbulence could have both direct and indirect consequences for the life cycle and population dynamics of the affected organisms.

The results of this experiment and the previous one on *A. sanguinea* (Berdalet 1992) suggest that the effects of turbulence can increase with cell size. The four dinoflagellate species studied were much smaller than the smallest turbulent eddies in the water, estimated to be between 370 and 1200 μm using the Kolmogorov length microscale (Tennekes and Lumley 1972). However, even below the Kolmogorov scale, cells could be affected by shear stresses, and these effects may increase with cell size (Lazier and Mann 1989). Indeed, the greatest growth inhibition was observed in the largest dinoflagellate studied (*A. sanguinea*), while the smallest one (*Gymnodinium* sp.) showed no significantly different growth rates under still and shaken conditions. In addition to size, shape could be a factor in the response to turbulence. For example, the two dorsoventrally flattened organisms, *A. sanguinea* and *P. triestinum*, showed the highest sensitivity to turbulence. Our experiments do not support the hypothesis that the absence of thecal plates would confer a special vulnerability to turbulence as suggested by other authors (Thomas et al. 1997). Further, our data indicate that the sensitivity to our experimentally generated turbulence was not linked to a particular taxonomic affiliation. As these trends have been derived from a rather small data set, they should not be interpreted as generalizations for the whole dinoflagellate domain, but as testable hypotheses for further studies and analyses. Nevertheless, our results agree with those of Sullivan and Swift (2003), who, using another experimental design, found no clear link between the sensitivity to small-scale turbulence and the size, shape, or taxonomy of 10 different dinoflagellates species.

Turbulence affected *A. sanguinea* (Berdalet 1992), *A. minutum*, and *P. triestinum* (Figs. 4 and 6) when it was applied during the exponential, rather than the stationary, phase. This finding means that turbulence affects these species while they are actively growing, a fact that could represent a potential impact during the formation of blooms. In contrast, Juhl and Latz (2002) observed that the growth of *L. polyedra* under shear stress in Couette cylinders ($\sim$0.18 $cm^2 \cdot s^{-3}$) was most inhibited during the stationary phase and concluded that turbulence was more important at terminating blooms. Again, these contradictory results suggest that the specificity of the response can also include the phase of the population development during which a species is sensitive to small-scale turbulence.

In ecophysiological studies, the use of stronger conditions than those typical in nature may help to ascertain the underlying mechanisms of cell adaptations. This work and that of Berdalet (1992) were

conducted using an orbital shaker at a relatively high intensity of ε (bulk average, $\sim$2.01 $cm^2 \cdot s^{-3}$; range, 0.27–24 $cm^2 \cdot s^{-3}$), which lasted for more than 3 d. These turbulence intensities can be generated in the upper few meters of coastal zones under high wind (20 $m \cdot s^{-1}$) conditions (Granata and Dickey 1991, MacKenzie and Leggett 1993, Kiørboe and Saiz 1995). Values of ε close to our experimentally generated range have been estimated during turbulence events derived from storms and frontal systems that have a 4–8 d periodicity in NW Mediterranean coastal waters (Guadayol and Peters 2006), while the background range of ε intensities would be 10^{-5} to 10^{-3} $cm^2 \cdot s^{-3}$.

Turbulence did not affect the small *Gymnodinium* sp. This finding agrees with the observations that small gymnodinioids are normally observed in the photic zone of coastal and oceanic waters of the NW Mediterranean Sea both during the stratification and mixing seasons (Estrada and Salat 1989, Estrada 1991, 1999). In contrast, blooms of *A. minutum* occur during calm waters in harbors of the NW Mediterranean Sea (Garcés et al. 1998, Vila et al. 2004).

Our results are in line with several other studies (Pollingher and Zemel 1981, Karentz 1983, Berdalet 1992, Thomas et al. 1995, Juhl et al. 2000, Yeung and Wong 2003, Yeung et al. 2006) that indicated a direct effect of turbulence on dinoflagellate cell-division and life-cycle processes, although species-specificity is increasingly evident (Berdalet and Estrada 1993, Juhl et al. 2001, Sullivan and Swift 2003) and the cytological and/or physiological mechanisms involved are still poorly known. The sensitivity of dinoflagellates to turbulence may be related to the peculiar division mechanism (dinomitosis) of their nucleus or dinokaryon (Karentz 1987, Berdalet 1992). Very recently, Yeung et al. (2006) proposed that mechanically induced cell-cycle arrest in the dinoflagellate *C. cohnii* involves the metabolism of calcium, which has a key role in connecting cellular signaling networks. Certainly, gaining insight into the cell-cycle control of dinoflagellates is an important aspect that will require the combination and improvement of different techniques. Among the possible methods are microfluorometry or flow cytometry with adequate fixation and staining procedures to detect changes in DNA content or cell damage or death; microscopic observations of the cellular forms in vivo to clarify the influence of turbulence on the (sexual and/or temporary) cyst induction; observation of the expression of different microtubular proteins involved in the dinomitosis process (Ausseil et al. 2000); ultrastructural studies (Soyer-Gobillard and Géraud 1992); and sophisticated pharmacological tools (Yeung et al. 2006).

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